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AL-RUBAYE, Ali Ghamin, NABOK, Alexei <<http://orcid.org/0000-0002-9078-1757>> and TSARGORODSKAYA, Anna

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Spectroscopic ellipsometry study of gold nanostructures for LSPR bio-sensing applications☆

Ali Ghamin Al-Rubaye^{a,*}, Alexei Nabok^a, Anna Tsargorodskaya^b

^a Sheffield Hallam University, Materials and Engineering Research Institute, UK

^b The University of Sheffield, Department of Chemistry, UK

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ABSTRACT

This main aim of this work is the development of optical biosensors based on the LSPR phenomenon in nano-structured gold films suitable for detection of low molecular weight analytes such as mycotoxins. A simple technology of annealing thin gold films was utilized for the formation of gold nano-islands exhibiting the LSPR effect. The morphology of gold nano-structures produced was studied with SEM and AFM, and their optical properties were analysed with UV-visible absorption spectroscopy and spectroscopic ellipsometry (SE). The position of LSPR band appeared to depend on the dimensions of gold nano-islands. The dependence of the LSPR band spectral shift on the refractive index of a medium was studied with both UV-vis absorption and SE, and the refractive index sensitivity (RIS) was evaluated. The method of SE gave from two to three times higher values of RIS as compared to those obtained by absorption spectroscopy. LSPR bio-sensing tests were attempted using total internal reflection ellipsometry (TIRE); a noticeable spectral shift was recorded on course of immune binding of Aflatoxin B1 to specific antibodies immobilized on the surface of gold.

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1. Introduction

The environmental pollution became a great concern in the modern industrial world. Thousands of different toxic chemicals of both natural and human-made origin are threatening the life on the planet. One specific type of pollution, e.g. mycotoxins, is of particular interest in this work. Mycotoxins being products of metabolism of fungi species are dangerous to human and animal health in connection with their hepato- and nephro- toxicity, carcinogenic, genotoxic and mutagenic actions [1]. Different agriculture products, particularly grains and grain related food, nuts, coffee beans, spices, and fruits are typical media for fungi growth at elevated temperature and high humidity. There were hundreds of fungi species which can produce mycotoxins, for example *Fusarium* fungi can produce T2 mycotoxin and zearalenone, while *Aspergillus* fungi can produce aflatoxin and ochratoxin, well-known as extremely dangerous mycotoxins [1]. Contamination of animal feed may also lead to penetration of mycotoxins into food chain via respective animal products, such as milk. Also, the relative ease of production of mycotoxins causes great security concern [2].

Development of biosensors for mycotoxins detection is in great demand nowadays, with optical immuno-sensors leading the way [2]. There are many types of optical transducers used in optical bio-sensing with surface plasmon resonance (SPR) being the most common and widely used [2,3]. The method of Total Internal Reflection Ellipsometry (TIRE) combines the advantages of SPR and spectroscopic ellipsometry and offer 10 times higher sensitivity than SPR and therefore became particularly suitable for detection of low molecular weight molecules such as mycotoxins [4]. Recent advances in nanotechnology brought to practice some new methods which explore a physical phenomenon of localized surface plasmon resonance (LSPR) [5]. In this work we are trying to study LSPR phenomenon using spectroscopic ellipsometry (SE) and to explore for the first time the use TIRE method for LSPR bio-sensing applications, particularly for detection of Aflatoxin B1.

2. Sample preparation and experimental methodology

Gold nanostructured, were prepared by evaporation of gold in 10^{-6} Torr vacuum onto standard microscopic glass slides using an intermediate 2 nm thick layer of chromium to improve adhesion of gold. Thin gold films of different nominal thicknesses (4, 5, 6, 8, and 10 nm) were deposited and then annealed at 480 °C for 2 h. For comparison, 5 nm thick Au films (with no Cr under-layer) were annealed at higher temperature (550 °C) for 10 h in order to form Au nano-islands embedded into glass matrix, similar to those reported in [6].

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* Corresponding author.

E-mail address: b3033014@my.shu.ac.uk (A.G. Al-Rubaye).

The morphology of samples of nano-structured gold films was studied first with SEM (FEI-Nova, NanoSEM 200) and AFM (Nanoscope IIIa, Bruker). Optical properties of gold nano-structures were studied with UV-visible absorption spectroscopy (Cary 50, Varian) and spectroscopic ellipsometry (M2000, J.A. Woollam). In order to evaluate the refractive index sensitivity of LSPR gold nanostructures structures produced, UV-vis absorption spectra and SE measurements were performed in media with different refractive indices: air ($n = 1$), water ($n = 1.3325$), ethanol ($n = 1.3616$), chloroform ($n = 1.4441$), and DMSO ($n = 1.4772$). For SE measurements, a special 70° PTF cell was designed.

Some of the spectroscopic ellipsometry (SE) measurements were carried out using total internal reflection ellipsometry (TIRE) configuration described in detail in our early publications [4,7,8]. In this work, the detection of mycotoxin aflatoxin B1 was carried out in direct immunoassay with specific monoclonal antibodies to aflatoxin B1 immobilized electrostatically on the surface of gold. The electrostatic immobilization of proteins which was developed and routinely used in our work previously [4,7,8] requires the following adsorption/binding stages: (i) overnight treatment of gold-coated samples in 0.1 M solution of sodium 3-mercaptopropyl-sulfonate in methanol which makes the surface of

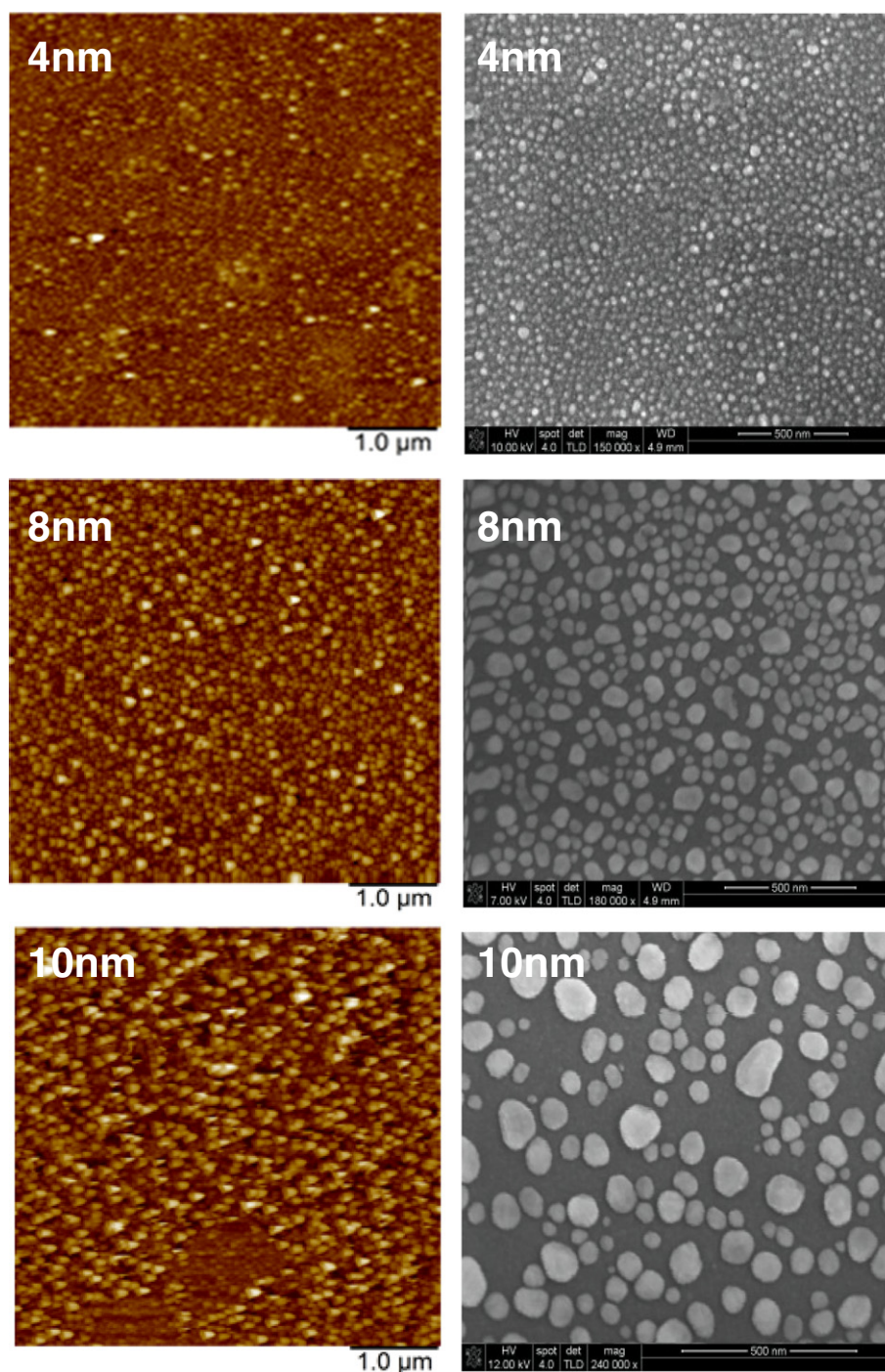


Fig. 1. Selection of typical AFM (left) and SEM (right) images of nano-structured gold films of different nominal thicknesses. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

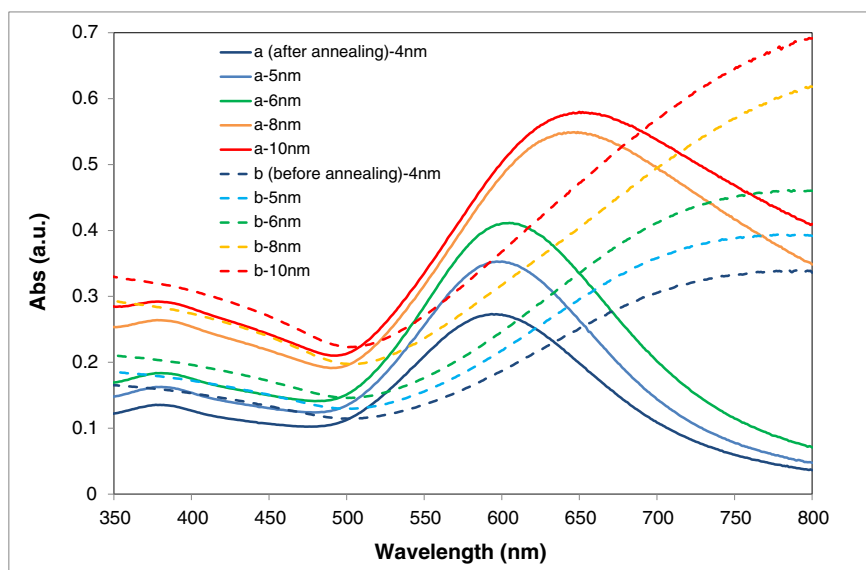


Fig. 2. UV-vis absorption spectra of Au thin films of different thicknesses before (dotted lines) and after (solid lines) thermal annealing.

Au negatively charged, (ii) deposition of polycation layer of poly(allylamine) hydrochloride (PAH) from its 1 mg/ml aqueous solution; (iii) deposition of protein A from its solution 0.02 mg/ml in Tris-HCl buffer, pH 7.5; deposition of antibodies to aflatoxin B1 from its 1:1000 solution in the same buffer; (iv) and finally sequential binding of aflatoxin B1 of different concentrations 1 ng/ml, 10 ng/ml, 100 ng/ml, and 1 μ g/ml in water starting from the smallest. Typical incubation time for all adsorption/binding stages was from 10 to 15 min. The use of protein A helps improving the orientation of IgG based antibodies

immobilized on the surface [4]; occasionally, for testing purposes the deposition protein A was skipped as not essential.

3. Experimental results and discussion

3.1. SEM and AFM study

Annealing of thin gold films causes the formation of well-defined Au nano-islands as shown in AFM and SEM images in Fig. 1. The nano-islands are visibly irregular in their dimensions and spatial arrangement, however, the mean size of Au nano-islands increases with the increase of the nominal thickness of Au films. Large Au nano-islands formed from 10 nm thick Au films are becoming faceted which demonstrate their high level of crystallographic order. The Au nano-islands formed at higher temperature 550 $^{\circ}$ C and longer annealing time (10h) are larger than those annealed at lower temperature and shorter time. Furthermore, they are partially embedded into the glass matrix as was shown in [9] which may provide better stability of nano-structured gold films in biosensing experiments.

3.2. Optical measurements

UV-vis absorption spectra in Fig. 2 showed a dramatic transition from classical Drude dispersion spectra for continuous (as deposited) gold films to distinctive LSPR band at around 600 nm for gold nano-islands as a result of thermal annealing. The position of LSPR band shifts to higher wavelength with the increase in the mean size of Au nano-islands which correlate with nominal film thickness. Ellipsometric spectra of Ψ and Δ^* in Fig. 3 showed characteristic features associated with LSPR phenomenon, i.e. the minima in the amplitude-related Ψ spectra between 600 nm and 700 nm and corresponding phase change in Δ spectra. Similarly to UV-vis absorption spectra, the position of LSPR features shift towards high wavelengths when the size of Au nano-islands increases. Also, LSPR effect is more pronounced in larger Au nano-islands. Ellipsometry data fitting, which was performed using dedicated

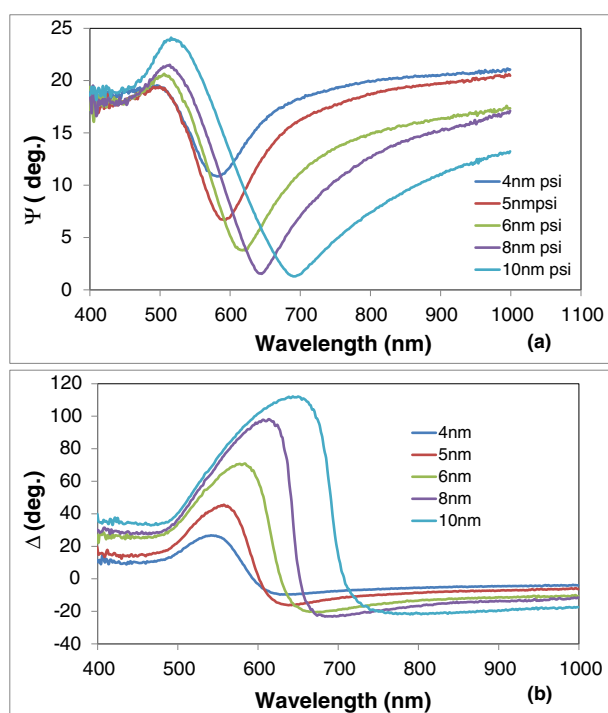


Fig. 3. Ellipsometry spectra of Ψ (a) and Δ (b) of Au nano-structured films of different nominal thicknesses.

* Ψ and Δ represent respectively the ratio of amplitudes and the phase shift between p- and s- components of polarized light; $m\Psi = A_p/A_s$, $\Delta = \varphi_p - \varphi_s$.

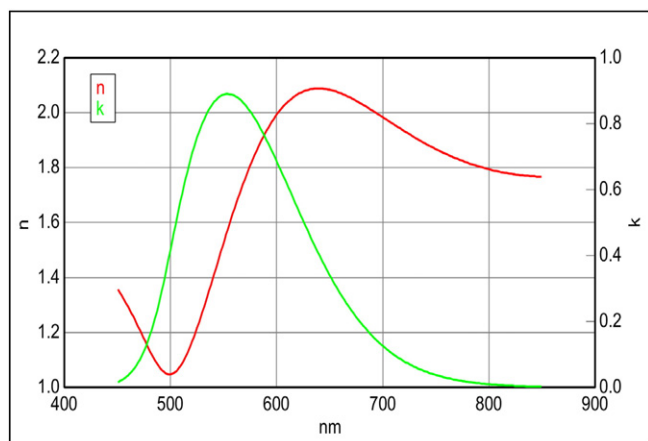


Fig. 4. Typical dispersion characteristics of n and k obtained by ellipsometry data fitting.

J.A. Woollam software, has resulted in dispersion characteristics of refractive index (n) and extinction coefficient (k), i.e. spectra of n and k , the example of which is shown in Fig. 4. As one can see, the peak in k spectrum resembles closely LSPR peak in absorption spectra in Fig. 3. The positions of LSPR band obtained from absorption spectra and k dispersions appeared to be very close to each other.

3.3. Evaluation of refractive index sensitivity

The main principle of LSPR sensing lies in the dependence of LSPR peak position on the refractive index of the surrounding medium. As

Table 1

The comparison of the refractive index sensitivity obtained from absorption spectra and SE measurements on nano-structured Au films.

Nominal thickness of Au films (nm)	RIS (nm/RIU) (abs. spectra)	RIS (nm/RIU) (ellipsometry)	RIS _{SE} /RIS _{Abs}
4	43.9	143.6	3.27
5	66.6	165.7	2.49
6	78.8	155.1	1.97
8	96.2	207.2	2.15

described in the previous section, a series of optical tests using UV-vis spectrophotometry and SE were performed in media having different refractive indices, and the results are presented in Fig. 5 as dependencies of LSPR band position vs. refractive index. All dependencies are linear and their gradients gave an important parameter of refractive index sensitivity (RIS). The obtained values of RIS are summarized in Table 1. There are two important findings to be highlighted: (i) RIS increases with the increase in the nominal thickness and thus the size of Au nano-islands, (ii) SE measurements gave higher RIS values as compared to that obtained by absorption spectroscopy (see the RIS_{SE}/RIS_{Abs} ratio in Table 1). The latter fact is surprising because the RIS must be related to physical parameters of Au nano-structured, not the methods of their analysis. This fact could be understood if we take into account multiple reflections of light in ellipsometry measurements. According to Fresnel's theory the number of reflections of light in ellipsometry of thin films is infinite, though only the first three reflections can be considered as significant [10]; that is perhaps why RIS in ellipsometry experiments is two to three times higher.

3.4. TIRE biosensing tests

A series of TIRE measurements of nano-structured gold films were performed and the results are reported here for the first time. As was described previously [4, 7, 8], the method of TIRE appeared as a combination of spectroscopic ellipsometry instrumentation and SPR Kretschman geometry [11]. In TIRE measurements (the schematic diagram is shown as inset in Fig. 6) the light is coupled into the gold-coated glass slides via 68° prism; the cell attached underneath to the gold side of the sample allows injecting liquids and thus performing bio-sensing tests. Typical TIRE spectra recorded in 25 nm thick continuous gold films deposited on glass slides are shown in Fig. 6. As one can see, the amplitude-related Ψ spectrum resembles SPR curve. The Δ spectrum show a characteristic phase drop near the resonance, position of which is very sensitive to small changes in refractive index or thickness of molecular layers adsorbed on gold surface. It was shown that the sensitivity of TIRE, e.g. Δ spectra, is 10 times higher than that of traditional SPR [4], and the method of TIRE was particularly effective for detection of small molecules of toxins including mycotoxins.

A series of TIRE spectra recorded on nano-structured Au films are shown in Fig. 7. The LSPR features, i.e. minima on Ψ spectra and phase drop in Δ spectra, are quite obvious. The increase in the size of Au nano-islands (or nominal Au film thickness) causes the shift of LSPR to lower wavelengths.

The results of biosensing tests on detection of aflatoxin B1 in direct immunoassay with specific antibodies are shown in Fig. 8. The samples of 5 nm Au annealed at high temperature of 550 °C appeared to be more stable than those annealed at lower temperature as therefore they were used in these measurements. As one can see, a noticeable spectral shift upon absorption of layers of PAH, antibodies, and aflatoxin B1 was observed. These results are similar to those obtained earlier on continuous gold films [12]. The spectral shift of nearly 20 nm caused by binding aflatoxin B1 from its stock solution (1 µg/ml in water) is particularly impressive.

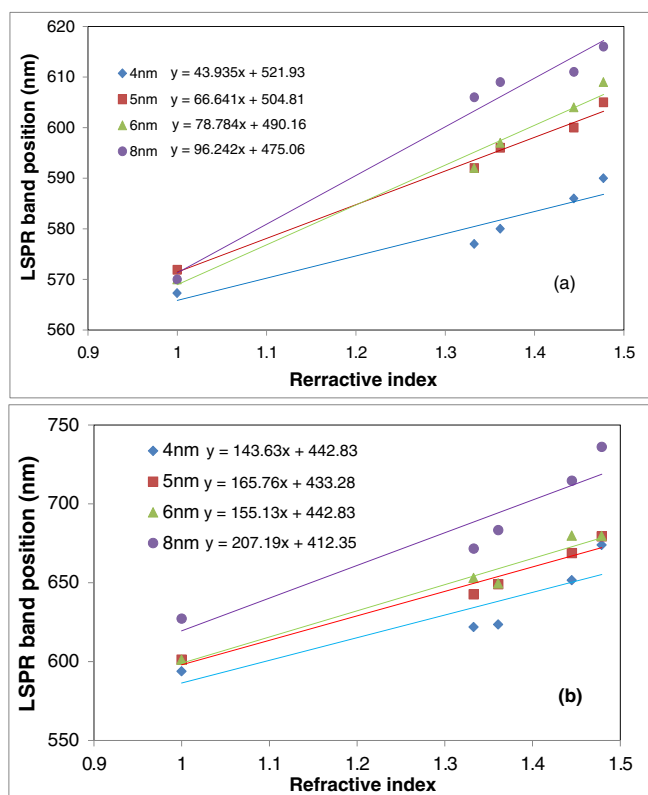


Fig. 5. Dependencies of LSPR band position vs. refractive index obtained from absorption spectra (a) and ellipsometry (b) measurements.

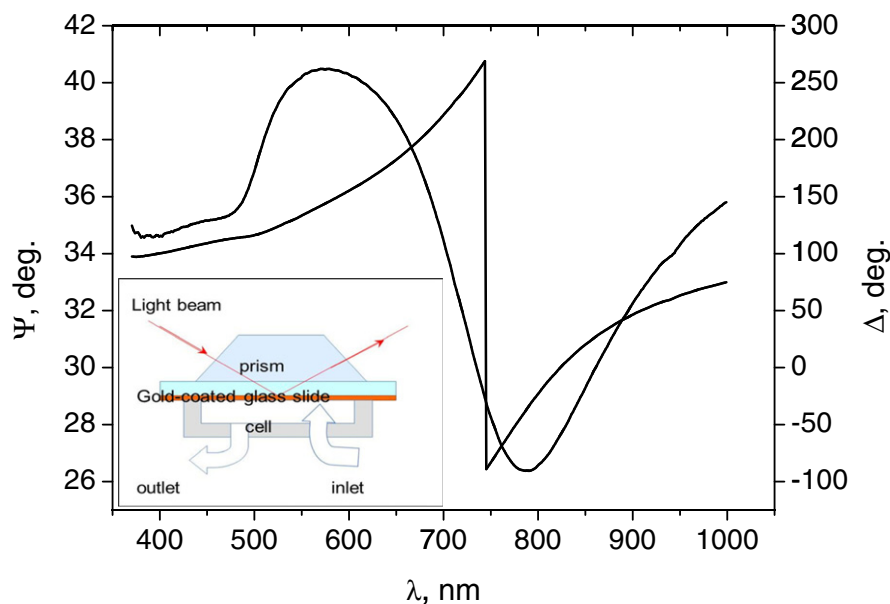


Fig. 6. Typical TIRE spectra of continuous 25 nm thick Au film. Inset shows the geometry of TIRE set-up.

4. Conclusions and suggestions for future work

Spectroscopic ellipsometry measurements of nano-structured gold films provided additional information for studying LSPR phenomenon. The data obtained correlated well with the traditional UV-vis absorption spectra as well as with the morphology of Au nano-islands.

The refractive index sensitivity appeared to depend on the size of Au nano-islands. Also spectroscopic ellipsometry showed two to three times higher refractive index sensitivity than that of absorption spectroscopy which is most-likely related to multiple reflections of light in ellipsometry measurements.

The method of TIRE was proved to be suitable for studying LSPR phenomenon in Au nanostructures. The immune binding of aflatoxin B1 to specific antibodies immobilized on the surface of gold resulted in substantial shift of Δ spectra.

Further work is currently underway, and it is focused on optimizing the technology for Au nano-structures fabrication, improving their stability, and repeating biosensing tests using different analytes and bio-receptors.

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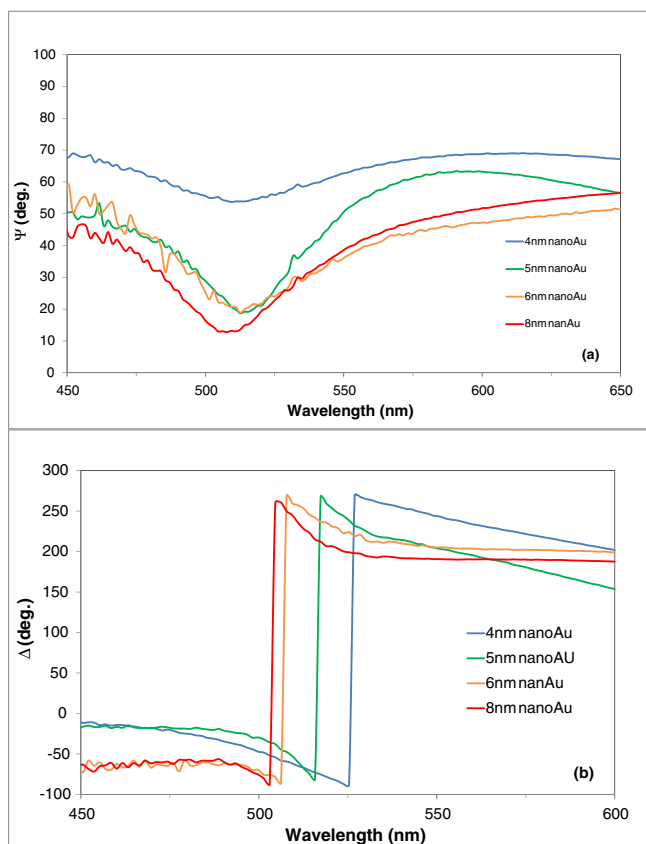


Fig. 7. TIRE spectra Ψ (a) and Δ (b) for Au nano-structures of different nominal thicknesses.

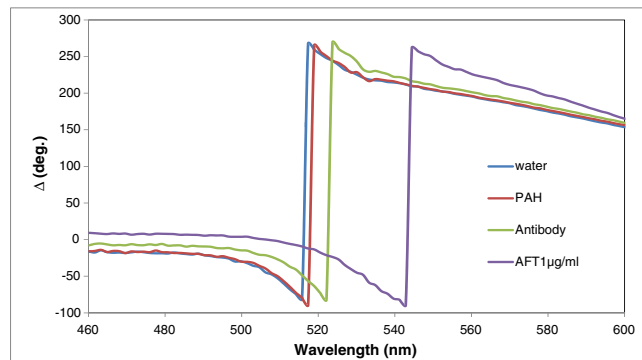


Fig. 8. The series of TIRE Δ spectra corresponding to deposition of layers of PAH, antibodies, and aflatoxin B1 on nanostructured Au film of 5 nm of nominal thickness annealed at higher temperature 550 °C for 10 h.

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